Intake of conjugated linoleic acid, fat, and other fatty acids in relation to postmenopausal breast cancer: the Netherlands Cohort Study on Diet and Cancer

Intake of conjugated linoleic acid, fat, and other fatty acids in relation to postmenopausal breast cancer: the Netherlands Cohort Study on Diet and Cancer[1–3]

Laura E Voorrips, Henny AM Brants, Alwine FM Kardinaal, Gerrit J Hiddink, Piet A van den Brandt, and R Alexandra Goldbohm

ABSTRACT

Background: Conjugated linoleic acid (CLA), which is present in milk products and meat from ruminants, appears to have anticarcinogenic activity against breast cancer in animal and in vitro experiments. To date, few epidemiologic data are available in humans.

Objective: This study evaluated the relation between intakes of CLA and other fatty acids and breast cancer incidence in the Netherlands Cohort Study.

Design: Intake data derived from a validated 150-item food-frequency questionnaire were linked to an existing database with analytic data on specific fatty acids in European foods (the TRANSFAIR study). With 6.3 y of follow-up and 941 incident cases of breast cancer, multivariate rate ratios and 95% CIs were calculated for energy-adjusted intakes of fatty acids and CLA-containing food groups (eg, butter, cheese, milk, other milk products, and meat).

Results: CLA intake showed a weak, positive relation with breast cancer incidence (rate ratio for highest compared with lowest quintile: 1.24, 95% CI: 0.91, 1.69; *P* for trend = 0.02). Statistically significant positive associations were found with total *trans* fatty acids and (borderline) with saturated fatty acids. Significant inverse associations were found with monounsaturated and *cis* unsaturated fatty acids, whereas total fat and energy intake of CLA-containing food groups were not related to breast cancer incidence.

Conclusion: The suggested anticarcinogenic property of CLA in animal and tissue culture models could not be confirmed in this epidemiologic study in humans. *Am J Clin Nutr* 2002;76:873–82.

KEY WORDS Cohort study, breast cancer, intake, fat, fatty acids, conjugated linoleic acid, *trans* fatty acids

INTRODUCTION

In the 1970s, breast cancer was associated with fat intake on the basis of national incidence and mortality rates for breast cancer and national per capita fat consumption (1). Since then, many epidemiologic studies focused on the relation between total fat intake and breast cancer. From reviews and pooled analyses it can be concluded that cohort studies present no evidence of a positive association between total dietary fat intake and breast cancer, but case-control studies occasionally do so (2–4).

Few studies that have examined the relation between intake of fat or fatty acids and the risk of breast cancer have addressed the role of *trans* fatty acids (TFAs; 5–9). Dietary TFAs predominantly derive from industrially hydrogenated vegetable and marine oils but are also found naturally in dairy and other animal fats. The major TFA in milk, butter, and beef fat is *trans* vaccenic acid (11*t*-18:1). A specific fatty acid with suggested anticarcinogenic properties is conjugated linoleic acid (CLA), which also is primarily found in dairy products and meat from ruminants and is hardly present in vegetable products. The anticarcinogenic and other properties of CLA were recently reviewed (10, 11). CLA is a collective term describing a mixture of positional and geometric conjugated diene isomers formed as intermediates in the biohydrogenation of linoleic acid to *trans* vaccenic acid or elaidic acid (9*t*-18:1) by anaerobic bacteria in the rumen of ruminants. The double bonds in CLA are conjugated—ie, contiguous—unlike the double bonds of linoleic acid, which are separated by a methylene group. The isomer most commonly occurring (> 80%) in dairy products is 9*c*,11*t*-18:2 (12). The anticarcinogenic activity of CLA has been shown in a wide range of animal models (13, 14) since 1979, when a lipid fraction of cooked ground beef was shown to have anticarcinogenic activity (15), and further characterized as a mixture of 4 CLA isomers (16). In vitro studies showed that physiologic concentrations of CLA inhibit the growth of human breast cancer cells in a dose-dependent manner (17–19). Animal experiments showed that CLA inhibits the formation of breast tumors in rats at dietary concentrations of 0.1–1.0% (by wt) (20). Concentrations of CLA higher than 1% appeared to have no additional protective effect (21). The protective action of CLA is independent of the diet's total fat content, the type of dietary fat (saturated or unsaturated) (22), or the concentration of linoleic

[1] From the Departments of Nutritional Epidemiology (LEV, HAMB, and RAG) and Nutritional Physiology (AFMK), TNO Nutrition and Food Research, Zeist, Netherlands; the Dutch Dairy Foundation for Nutrition and Health, Utrecht, Netherlands (GJH); and the Department of Epidemiology, Maastricht University, Maastricht, Netherlands (PAVDB).

[2] Supported by the Dutch Dairy Foundation for Nutrition and Health.

[3] Address reprint requests to LE Voorrips, Department of Nutritional Epidemiology, TNO Nutrition and Food Research, PO Box 360, 3700 AJ Zeist, Netherlands. E-mail: voorrips@voeding.tno.nl.

Received June 5, 2001.

Accepted for publication November 13, 2001.

acid in the diet (21). Supplementation of CLA to rats during the developmental stage of the mammary gland only was found to confer lifelong protection against breast tumors (20). CLA concentrations in breast tissue relate inversely to the rate of formation of new tumors after the administration of a carcinogen to adult rats (23). Different CLA concentrations in the diets of mice (0.1%, 0.3%, or 0.9%) were found to exert specific effects on the immune system but had no effect on tumor growth and tumor volume after infusion with metastasizing breast cancer cells (24). Possibly CLA selectively inhibits the growth of estrogen receptor–positive breast cancer cells—a relation found in vitro (25).

CLA intake in Germany was estimated at 0.35 g/d for women and 0.43 g/d for men (12). CLA concentrations in milk and dairy products vary considerably by a factor of up to 10 in studies in which large numbers of samples are analyzed. Because CLA concentrations are dependent on feed composition and use of supplements, seasonal fluctuations in CLA concentrations are seen. On average, CLA concentrations in milk and dairy products range from 0.2 to 1.6 g/100 g fat (12, 26–28).

On the in vivo relation between CLA intake or tissue concentrations and risk of human breast cancer, few data have been published, from case-control studies only, with contradictory results (29–32). Within the framework of the Netherlands Cohort Study on Diet and Cancer (NLCS), we used data on CLA concentrations and other fatty acids in Dutch food products derived from the TRANSFAIR study (33). With the use of these data, previous analyses of total fat and several types of fat and breast cancer incidence (34) were updated and extended with individual fatty acids, including CLA and the very-long-chain n−3 polyunsaturated fatty acids (fish $n-3$ PUFAs), which also have suggested anticarcinogenic properties (35).

SUBJECTS AND METHODS

The Netherlands Cohort Study

The NLCS is a prospective cohort study that began in September 1986. The study design was reported in detail elsewhere (36). In brief, the cohort included 62 573 women aged 55–69 y at the beginning of the study, originating from 204 municipalities with computerized population registries. A self-administered mailed questionnaire on dietary habits, lifestyle, smoking, personal and family history of cancer, and demographic data was completed at baseline.

Accumulation of person-time in the cohort has been estimated by biennial follow-up for vital status information of a subcohort of 1812 women, randomly selected after baseline exposure measurement. No subcohort members were lost to follow-up. The study was approved by the Institutional Review Boards of TNO Nutrition and Food Research and of Maastricht University.

Identification of cases of breast cancer

The method of record linkage to obtain information on cancer incidence was described previously (37). In short, follow-up for incident cancer was established by computerized record linkage for the entire cohort with all regional cancer registries in the Netherlands and with the Dutch national database of pathology reports. Completeness of cancer follow-up was at least 96% (38).

The food-frequency questionnaire

The dietary section of the questionnaire was a 150-item semiquantitative food-frequency questionnaire. The questionnaire concentrated on the habitual consumption of food and beverages during the year preceding the start of the study. Among the principal dietary variables were energy and fat intake. Questionnaire data were key-entered twice and processed for all incident cases and subcohort members in a manner blinded with respect to case and subcohort status. This was done to minimize observer bias in coding and interpretation of the data. Main nutrient intakes were calculated with the use of the Dutch food composition table (39). Intakes of specific fatty acids were calculated from a separate database derived from the TRANSFAIR study. This was a market basket study in 14 European countries in each of which a maximum of 100 foods contributing most to the total fat intake of the country were sampled and analyzed as methyl esters of the fatty acids present in the foods. All analyses were performed in one central laboratory. In the database, total fat included triacylglycerol and other lipids, such as phospholipids and sterols. The percentage of triacylglycerol in total fat is assumed to be 93% on average, but it varies across food sources (33).

The food-frequency questionnaire was validated against a 9-d diet record. Pearson correlation coefficients between the dietary record and the questionnaire varied from 0.40 for vitamin B1 to 0.86 for alcohol intake, with a median of 0.69. For the exposure variables under study crude (and energy and sex adjusted) Pearson correlation coefficients were as follows: energy, 0.74; total fat, 0.72 (0.52); saturated fat, 0.73 (0.58); and polyunsaturated fat, 0.73 (0.75) (40).

Population for analysis

Subjects who reported prevalent cancer at baseline other than nonmelanoma skin cancer, subjects without microscopically confirmed cancer, and subjects with incident in situ carcinoma were excluded. From the subcohort, prevalent cancer cases other than nonmelanoma skin cancer were excluded as well. Subjects with incomplete or inconsistent dietary data were excluded from analyses. Criteria for exclusion were *1*) subjects who left blank ≥ 60 (of 150) items in the questionnaire and reported eating < 35 items ≥ 1/mo or *2*) subjects who left one or more item blocks (grouping of items, eg, beverages) blank. More details are given in a separate report (40). In the first 6.3 y of follow-up (September 1986–December 1992), 941 incident breast cancer cases and 1598 subcohort members were left for analyses.

Data analysis

In the present analysis, exposure variables included intake of total energy, total fat, animal fat, vegetable fat, total fatty acids, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, *trans* unsaturated fatty acids, *cis* unsaturated fatty acids, and specific fatty acids including palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidonic acid, and the fish $n-3$ PUFAs eicosapentaenoic acid and docosahexaenoic acid. For CLA, the total of the 9*c*,11*t* and 9*t*,11*c* isomers is used. The TRANSFAIR data on 18:1 *trans*-isomers were roughly separated into "predominantly *trans* vaccenic acid" and "other 18:1 *trans*- isomers" based on food sources. To do so, the 18:1 *trans*-isomers in fat, meat, or milk from ruminants have been assumed to be predominantly *trans* vaccenic acid, whereas 18:1 *trans*-isomers from other sources were considered to be low in *trans* vaccenic acid. Fat intake was adjusted for energy intake (41).

Other exposure variables were intake of CLA-containing food groups: milk and milk products [categorized as whole milk (and products), semiskim milk (and products), fermented whole milk (and products), and fermented semiskim milk (and products)], cheese, fresh meat, and processed meat. Subjects were classified into quintiles or categories of consumption (g/d), based on the distribution in the

TABLE 1
Mean energy and energy-adjusted fat intake in breast cancer cases and female subcohort members in the Netherlands Cohort Study, 1986–1992[1]

	Cancer cases (n = 941)	Subcohort members (n = 1598)
Energy (kJ)	7057 ± 1670	7055 ± 1715
Fat (g/d)		
Total[2]	74.0 ± 10.8	74.0 ± 10.5
Animal	53.1 ± 14.8	52.8 ± 14.7
Vegetable	20.9 ± 13.0	21.2 ± 13.6
Fatty acids (g/d)		
Total[3]	68.5 ± 10.3	68.6 ± 10.1
Total saturated	29.5 ± 6.7	29.1 ± 6.2
Total monounsaturated	22.1 ± 4.2	22.3 ± 4.2
Total polyunsaturated	15.4 ± 6.4	15.6 ± 6.3
Total *trans* unsaturated	2.5 ± 0.9	2.5 ± 0.9
Total *cis* unsaturated	34.9 ± 7.7	35.4 ± 7.7
Palmitic acid, 16:0	14.7 ± 2.9	14.7 ± 2.8
Stearic acid, 18:0	6.7 ± 1.2	6.6 ± 1.2
Oleic acid, 9*c*-18:1	16.8 ± 3.5	16.9 ± 3.3
Linoleic acid, 9*c*,12-18:2	13.5 ± 6.3	13.8 ± 6.2
Conjugated linoleic acid, 18:2 (9*c*,11*t* or 9*t*,11*c*)	0.2 ± 0.1	0.2 ± 0.1
trans Vaccenic acid, 11*t*-18:1	0.8 ± 0.4	0.7 ± 0.4
Other 18:1 *trans*-isomers, 6*t* or 9*t*	1.2 ± 0.8	1.2 ± 0.8
Linolenic acid, 9*c*,12,15-18:3	1.0 ± 0.4	1.1 ± 0.5
Arachidonic acid, 5*c*,5,8,11,14-20:4	0.10 ± 0.04	0.10 ± 0.04
Eicosapentaenoic acid, 5*c*,8,11,14,17-20:5	0.03 ± 0.04	0.03 ± 0.04
Docosahexaenoic acid, 4*c*,7,10,13,16,19-22:6	0.07 ± 0.06	0.06 ± 0.07

[1] $\bar{x}$ ± SD.
[2] Includes other lipids such as phospholipids.
[3] Includes ≈2% unidentified fatty acids.

subcohort. In general, categories were used instead of quintiles when a large proportion of subjects were nonusers or when for another reason distributions in approximately equal quintiles led to problems. Analyses were performed with the case-cohort approach (42): cases were enumerated for the entire cohort, and the person-years at risk of the entire cohort were estimated for the subcohort sample.

Rate ratios (RRs) and 95% CIs were computed for quintiles or categories of intake with the statistical package STATA (43). Exponentially distributed survival times were assumed in the follow-up period. Tests for trend in RRs were based on two-sided likelihood ratio tests. Both age-adjusted and multivariate analyses were conducted. To enable comparison, age-adjusted analyses were restricted to subjects included in multivariate analyses (eg, with no missing values on confounders included in the multivariate model). As in our earlier publication, confounders adjusted for in the multivariate analyses were age, history of benign breast disease, maternal breast cancer, breast cancer in one or more sisters, age at menarche, age at menopause, oral contraceptive use, parity, age at first childbirth, Quetelet index, education, alcohol use, current cigarette smoking, total energy intake, and total energy-adjusted fat intake. The addition of energy-adjusted fat to the model was performed to study the independent effect of each individual fatty acid (or fat subgroup) on breast cancer incidence by substituting it for any other type of fat (34). To eliminate the effect of potential undiagnosed disease on fat intake, analyses were repeated excluding (arbitrarily) those cases that were diagnosed during the first 2 y of follow-up.

TABLE 2
Daily consumption of food groups that contain conjugated linoleic acid in breast cancer cases and subcohort members in the Netherlands Cohort Study, 1986–1992[1]

	Cancer cases (n = 941)	Subcohort members (n = 1598)
	g	
Milk and milk products	296 ± 190	296 ± 186
Whole	88 ± 117	89 ± 111
Skim	90 ± 127	88 ± 126
Fermented whole	17 ± 42	19 ± 44
Fermented skim	101 ± 119	100 ± 122
Cheese	23 ± 19	23 ± 19
Fresh meat	93 ± 42	93 ± 40
Beef	26 ± 25	24 ± 21
Pork	34 ± 28	36 ± 29
Processed meat	11 ± 12	11 ± 13

[1] $\bar{x}$ ± SD.

RESULTS

In **Table 1** mean intakes of energy and energy-adjusted fat and fatty acids are presented for breast cancer cases and subcohort members. Differences in energy and nutrient intakes were very small, as was the case with intakes of CLA-containing product groups: milk and milk products, cheese, and fresh or processed meat (**Table 2**). Mean intake of CLA was 0.2 g/d, 29% of which originated from butter, 21% from cheese, and 19% from milk and milk products (**Table 3**). Twenty-four percent of CLA came from meat, particularly beef and ground meat.

TABLE 3
Sources of conjugated linoleic acid (CLA) contribution of food groups, subgroups, and individual food items to total CLA consumption in female subcohort members in the Netherlands Cohort Study, 1986–1992

Food group	Contribution
	%
Oils, fats, and sauces	29.6
Butter	29.3
Fresh meat and poultry	23.6
Beef	9.5
Beef steak	2.6
Veal	0.3
Other beef	6.7
Pork	3.6
Ground meat	9.1
Ground beef	5.4
Ground beef and pork	3.7
Chicken, turkey, other poultry	0.8
Other meats	0.6
Cheese	20.8
High-fat	20.0
Low-fat	0.8
Milk and milk products	19.4
Whole	10.4
Skim	5.3
Fermented whole	2.3
Fermented skim	1.4
Processed meat	2.5
Other food groups	4.1

Age-adjusted and multivariate RRs of breast cancer incidence were calculated for quintiles of energy and energy-adjusted fat intake (**Table 4**). Multivariate adjusted RRs were essentially the same as age-adjusted risk estimates, except that for monounsaturated fatty acids the multivariate RR was substantially lower than the age-adjusted RR. In multivariate analyses, no statistically significant trends were found for associations between breast cancer incidence and energy intake, intake of fat, vegetable fat, animal fat, total fatty acids, and total polyunsaturated fatty acids. Multivariate RRs for the highest compared with the lowest quintile were between 1.02 and 1.05 for energy intake and for vegetable and animal fat. For total fat and total fatty acids, the RRs were 1.16 (95% CI: 0.87, 1.56) and 1.13 (95% CI: 0.84, 1.52), respectively. For polyunsaturated fatty acids, the RR was 0.88 (95% CI: 0.65, 1.21).

Higher breast cancer incidence was observed with higher intake of *trans* unsaturated fatty acids (multivariate RR for the highest compared with the lowest quintile: 1.30, 95% CI: 0.93, 1.80; *P* for trend = 0.01), and borderline with saturated fatty acids (RR: 1.40, 95% CI: 0.97, 2.03; *P* for trend = 0.11). Inverse associations were found for total monounsaturated fatty acids and *cis* unsaturated fatty acids (RRs for the highest compared with the lowest quintile: 0.61, 95% CI: 0.38, 0.96; *P* for trend = 0.001, and 0.79, 95% CI: 0.54, 1.17; *P* for trend = 0.04, respectively).

In **Table 5**, RRs for specific fatty acids are presented. Statistically significant trends with breast cancer incidence were not found for most fatty acids. For palmitic acid, stearic acid, linoleic acid, other 18:1 isomers, arachidonic acid, and the fish $n-3$ PUFAs eicosapentaenoic acid and docosahexaenoic acid, multivariate RRs for the highest compared with the lowest quintile were between 0.89 and 1.01. Statistically significant positive trends were observed for CLA and *trans* vaccenic acid (multivariate RR for the highest compared with the lowest quintile: 1.24, 95% CI: 0.91, 1.69; *P* for trend = 0.02, and 1.34, 95% CI: 0.98, 1.82; *P* for trend = 0.006, respectively). Statistically significant negative trends were observed for oleic acid and linolenic acid (multivariate RR for the highest compared with the lowest quintile: 0.67, 95% CI: 0.44, 1.03; *P* for trend = 0.001, and 0.70, 95% CI: 0.51, 0.97; *P* for trend = 0.006, respectively).

On the basis of multivariate analyses, breast cancer incidence seemed not to be related to intake of milk and milk products or to specific subgroups of milk products, based on fat content or fermentation (**Table 6**). No associations were found for cheese, fresh meat, beef, or processed meat. A statistically significant positive trend with butter, the major source of CLA, was only present in the age-adjusted analysis but was less apparent when multivariate analysis was used. Intake of pork showed a statistically significant negative trend with breast cancer incidence, with a multivariate RR for the highest compared with the lowest quintile of 0.80 (95% CI: 0.60, 1.08) and a *P* for trend of 0.02.

DISCUSSION

The present study has almost twice the number of cases of our previous publication, issued after 3.3 y of follow-up (34), and conclusions drawn then can be grossly confirmed. Again, no relation was observed between intake of energy, total fat, or polyunsaturated fat and breast cancer incidence. In line with earlier results, intake of saturated fat showed a slightly positive association with breast cancer incidence, with a similar RR, but was not significant in the present analysis. With respect to monounsaturated fat, RRs for the upper compared with the lower quintile of intake were slightly lower (0.61 compared with 0.75 in the earlier analysis) and the trend was now significant. The multivariate RR for monounsaturated fatty acids was substantially lower than the age-adjusted RR. The main factor responsible is the adjustment for energy-adjusted total fat intake. In other words, substituting monounsaturated fat consumption for any other fat is related to a decrease in breast cancer incidence.

With the opportunity to link the TRANSFAIR database on fatty acids (33) to food consumption data and breast cancer incidence of the NLCS, we were able to study the potential protective effect of CLA on breast cancer incidence in postmenopausal women. CLA intakes were slightly lower than those estimated for Germany (based on analysis of CLA in 139 foods) (12) and approximately equal to the estimated intake in 123 Swedish men (44). Remarkably, a higher intake of CLA appeared to be associated with a slight increase in risk of breast cancer. The observed positive trend is in contrast to experiments in animals and in in vitro studies, where CLA appears to have protective properties against breast cancer. Although an important difference is that most animal studies have been performed with a free fatty acid preparation containing a mixture of CLA isomers, one study showed that high-CLA butter fat (with predominantly the 9*c*,11*t* isomer present in triacylglycerols) has anticarcinogenic properties in rats (45). It has been suggested that the timing of CLA provision might be important (46). In rats, adding CLA to the diet from the early postweaning to the pubertal period only (corresponding to active morphologic development of the mammary gland to the mature state) was sufficient to reduce subsequent induced tumorigenesis later in life. When CLA was not present in the diet in this period, but only after induction of tumorigenesis, inhibition of tumorigenesis was only maximal if CLA was added continuously afterward (46). Because the NLCS deals only with data on postmenopausal women, no data are available on CLA intake in the prepubertal period, though this might be a more important determinant of later tumor development (46).

A positive trend similar to that for CLA was found for *trans* vaccenic acid, but because intakes of both fatty acids are highly correlated (Pearson correlation coefficient = 0.95), their effects cannot be separated.

The major sources of CLA in the human diet—milk and milk products and meat from ruminants—showed no relation with breast cancer incidence. Two other longitudinal studies found no relation with milk consumption either (47, 48), but other prospective studies have reported inverse (49–51) or positive (52) associations. In a meta-analysis of cohort and case-control studies, high consumption of milk and cheese was associated with a very small increase in breast cancer risk (53). In a recent review, 3 case-control studies reported a negative association, 5 showed no association, and 5 a positive association between milk intake and breast cancer (54). Other studies found that lower risk of breast cancer was associated with higher intakes of whole milk but not of low-fat milk (55). As the World Cancer Research Fund summarized in its report in 1997 (2), data on the relation between meat intake and breast cancer incidence are inconclusive, with 3 of 8 cohort studies reporting a positive association and the remaining 5 reporting no association [including our previous paper (34)].

The effect of CLA on human breast cancer may be modified by other dietary factors, such as fiber, leaving the possibility that CLA may have different effects when it is part of different food patterns. In general, results should be considered as a contribution

TABLE 4
Rate ratios (RRs) and 95% CIs for breast cancer according to quintiles (Q) of intake of energy and energy-adjusted fat in the Netherlands Cohort Study, 1986–1992

Exposure	Q1[1]	Q2	Q3	Q4	Q5	*P* for trend
Energy intake						
Median intake in subcohort (kJ/d)	5079	6072	6900	7782	9247	
No. of cases	139	152	183	158	151	
RR[2] (95% CI)	1.00	1.09 (0.82, 1.45)	1.34 (1.02, 1.77)	1.13 (0.85, 1.51)	1.04 (0.78, 1.38)	0.68
RR[3] (95% CI)	1.00	1.05 (0.78, 1.43)	1.28 (0.95, 1.72)	1.13 (0.84, 1.53)	1.04 (0.77, 1.40)	0.61
Total fat						
Median intake in subcohort (g/d)	61	69	74	79	86	
No. of cases	166	145	162	143	180	
RR[2] (95% CI)	1.00	0.83 (0.65, 1.08)	0.95 (0.74, 1.22)	0.84 (0.65, 1.08)	1.05 (0.82, 1.34)	0.66
RR[4] (95% CI)	1.00	0.97 (0.71, 1.31)	1.11 (0.83, 1.50)	0.96 (0.71, 1.30)	1.16 (0.87, 1.56)	0.23
Vegetable fat						
Median intake in subcohort (g/d)	5	13	21	28	38	
No. of cases	161	162	156	148	156	
RR[2] (95% CI)	1.00	0.93 (0.71, 1.22)	0.96 (0.72, 1.26)	0.87 (0.66, 1.15)	1.01 (0.76, 1.34)	0.83
RR[4] (95% CI)	1.00	0.96 (0.71, 1.31)	0.98 (0.73, 1.33)	0.88 (0.65, 1.20)	1.02 (0.75, 1.38)	0.85
Animal fat						
Median intake in subcohort (g/d)	35	45	52	60	71	
No. of cases	168	155	149	139	172	
RR[2] (95% CI)	1.00	0.94 (0.71, 1.24)	0.88 (0.69, 1.16)	0.82 (0.62, 1.09)	1.01 (0.77, 1.33)	0.71
RR[4] (95% CI)	1.00	0.90 (0.67, 1.22)	0.91 (0.67, 1.24)	0.78 (0.58, 1.06)	1.05 (0.79, 1.40)	0.87
Total fatty acids						
Median intake in subcohort (g/d)	56	64	69	73	80	
No. of cases	154	147	163	151	168	
RR[2] (95% CI)	1.00	0.96 (0.73, 1.28)	1.11 (0.84, 1.46)	1.00 (0.76, 1.34)	1.08 (0.82, 1.43)	0.55
RR[4] (95% CI)	1.00	1.04 (0.77, 1.41)	1.18 (0.88, 1.60)	1.07 (0.79, 1.45)	1.13 (0.84, 1.52)	0.29
Saturated fatty acids						
Median intake in subcohort (g/d)	22	26	29	32	38	
No. of cases	140	161	152	142	188	
RR[2] (95% CI)	1.00	1.14 (0.86, 1.51)	1.07 (0.80, 1.42)	1.01 (0.76, 1.35)	1.31 (0.99, 1.72)	0.08
RR[5] (95% CI)	1.00	1.17 (0.86, 1.60)	1.12 (0.81, 1.56)	1.02 (0.72, 1.46)	1.40 (0.97, 2.03)	0.11
Total monounsaturated fatty acids						
Median intake in subcohort (g/d)	18	20	22	24	27	
No. of cases	168	169	164	142	140	
RR[2] (95% CI)	1.00	1.02 (0.78, 1.34)	0.97 (0.74, 1.28)	0.84 (0.63, 1.11)	0.89 (0.65, 1.15)	0.06
RR[5] (95% CI)	1.00	0.91 (0.66, 1.25)	0.82 (0.59, 1.15)	0.66 (0.45, 0.98)	0.61 (0.38, 0.96)	0.001
Total polyunsaturated fatty acids						
Median intake in subcohort (g/d)	8	12	15	18	24	
No. of cases	194	136	152	135	166	
RR[2] (95% CI)	1.00	0.65 (0.50, 0.86)	0.77 (0.59, 1.02)	0.72 (0.54, 0.95)	0.88 (0.67, 1.15)	0.40
RR[5] (95% CI)	1.00	0.70 (0.52, 0.95)	0.85 (0.63, 1.15)	0.73 (0.53, 1.00)	0.88 (0.65, 1.21)	0.39
trans Unsaturated fatty acids						
Median intake in subcohort (g/d)	1.5	2.1	2.4	2.8	3.6	
No. of cases	145	149	153	169	167	
RR[2] (95% CI)	1.00	1.00 (0.75, 1.33)	1.06 (0.80, 1.41)	1.17 (0.89, 1.55)	1.20 (0.90, 1.58)	0.04
RR[5] (95% CI)	1.00	0.98 (0.72, 1.33)	1.09 (0.80, 1.49)	1.20 (0.88, 1.64)	1.30 (0.93, 1.80)	0.01
cis Unsaturated fatty acids						
Median intake in subcohort (g/d)	26	31	35	39	45	
No. of cases	167	178	150	144	144	
RR[2] (95% CI)	1.00	1.03 (0.79, 1.35)	0.92 (0.70, 1.22)	0.89 (0.67, 1.18)	0.90 (0.68, 1.20)	0.16
RR[3] (95% CI)	1.00	1.10 (0.81, 1.49)	0.92 (0.66, 1.29)	0.89 (0.63, 1.26)	0.79 (0.54, 1.17)	0.04

[1] Reference category.

[2] Adjusted for age.

[3] Adjusted for age, history of benign breast disease, maternal breast cancer, breast cancer in one or more sisters, age at menarche, age at menopause, oral contraceptive use, parity, age at first childbirth, Quetelet index, education, alcohol use, and current cigarette smoking.

[4] Adjusted for age, history of benign breast disease, maternal breast cancer, breast cancer in one or more sisters, age at menarche, age at menopause, oral contraceptive use, parity, age at first childbirth, Quetelet index, education, alcohol use, current cigarette smoking, and total energy intake.

[5] Adjusted for age, history of benign breast disease, maternal breast cancer, breast cancer in one or more sisters, age at menarche, age at menopause, oral contraceptive use, parity, age at first childbirth, Quetelet index, education, alcohol use, current cigarette smoking, total energy intake, and total energy-adjusted fat intake.

TABLE 5
Rate ratios (RRs) and 95% CIs for breast cancer according to quintiles (Q) of intake of specific energy-adjusted fatty acids in the Netherlands Cohort Study, 1986–1992

Exposure	Q1[1]	Q2	Q3	Q4	Q5	*P* for trend
Palmitic acid						
Median intake in subcohort (g/d)	11	13	15	16	18	
No. of cases	149	152	169	153	160	
RR[2] (95% CI)	1.00	0.98 (0.74, 1.31)	1.15 (0.87, 1.53)	1.03 (0.78, 1.37)	1.04 (0.78, 1.37)	0.63
RR[3] (95% CI)	1.00	1.00 (0.72, 1.35)	1.14 (0.83, 1.57)	1.00 (0.71, 1.42)	1.01 (0.68, 1.52)	0.89
Stearic acid						
Median intake in subcohort (g/d)	5.2	6.0	6.6	7.2	8.0	
No. of cases	145	153	172	157	156	
RR[2] (95% CI)	1.00	1.06 (0.80, 1.41)	1.18 (0.90, 1.56)	1.10 (0.83, 1.46)	1.04 (0.79, 1.38)	0.66
RR[3] (95% CI)	1.00	1.04 (0.76, 1.43)	1.13 (0.81, 1.57)	1.02 (0.71, 1.47)	0.93 (0.61, 1.41)	0.65
Oleic acid						
Median intake in subcohort (g/d)	13	15	17	18	21	
No. of cases	159	188	152	144	140	
RR[2] (95% CI)	1.00	1.19 (0.91, 1.56)	0.97 (0.73, 1.28)	0.88 (0.67, 1.17)	0.92 (0.69, 1.22)	0.06
RR[3] (95% CI)	1.00	1.09 (0.80, 1.49)	0.85 (0.60, 1.20)	0.71 (0.49, 1.05)	0.67 (0.44, 1.03)	0.001
Linoleic acid						
Median intake in subcohort (g/d)	7	10	13	16	22	
No. of cases	179	149	150	138	167	
RR[2] (95% CI)	1.00	0.77 (0.58, 1.01)	0.84 (0.63, 1.11)	0.77 (0.58, 1.02)	0.95 (0.72, 1.24)	0.68
RR[3] (95% CI)	1.00	0.84 (0.62, 1.14)	0.92 (1.68, 1.24)	0.80 (0.58, 1.10)	0.96 (0.71, 1.31)	0.67
Conjugated linoleic acid						
Median intake in subcohort (g/d)	0.07	0.11	0.14	0.19	0.29	
No. of cases	127	149	156	175	176	
RR[2] (95% CI)	1.00	1.13 (0.85, 1.51)	1.19 (0.89, 1.59)	1.31 (0.98, 1.74)	1.34 (1.01, 1.79)	0.005
RR[3] (95% CI)	1.00	1.09 (0.79, 1.49)	1.14 (0.83, 1.57)	1.29 (0.95, 1.76)	1.24 (0.91, 1.69)	0.02
trans Vaccenic acid						
Median intake in subcohort (g/d)	0.3	0.5	0.7	0.9	1.2	
No. of cases	124	155	147	179	178	
RR[2] (95% CI)	1.00	1.19 (0.89, 1.59)	1.17 (0.87, 1.57)	1.35 (1.01, 1.79)	1.40 (1.05, 1.86)	0.002
RR[3] (95% CI)	1.00	1.16 (0.84, 1.59)	1.19 (0.87, 1.62)	1.36 (1.00, 1.85)	1.34 (0.98, 1.82)	0.006
Other 18:1 isomers						
Median intake in subcohort (g/d)	0.4	0.8	1.1	1.5	2.3	
No. of cases	180	134	164	160	145	
RR[2] (95% CI)	1.00	0.75 (0.56, 0.99)	0.90 (0.69, 1.18)	0.93 (0.71, 1.22)	0.83 (0.63, 1.09)	0.44
RR[3] (95% CI)	1.00	0.80 (0.59, 1.09)	1.01 (0.75, 1.38)	1.01 (0.74, 1.37)	0.89 (0.65, 1.21)	0.91
Linolenic acid						
Median intake in subcohort (g/d)	0.6	0.8	1.0	1.3	1.7	
No. of cases	194	145	187	133	124	
RR[2] (95% CI)	1.00	0.76 (0.58, 1.00)	0.92 (0.71, 1.20)	0.69 (0.52, 0.91)	0.68 (0.51, 0.91)	0.001
RR[3] (95% CI)	1.00	0.78 (0.57, 1.05)	1.03 (0.76, 1.39)	0.74 (0.54, 1.00)	0.70 (0.51, 0.97)	0.006
Arachidonic acid						
Median intake in subcohort (g/d)	0.05	0.07	0.09	0.11	0.15	
No. of cases	168	153	147	152	163	
RR[2] (95% CI)	1.00	0.85 (0.64, 1.12)	0.90 (0.68, 1.19)	0.88 (0.67, 1.16)	1.01 (0.77, 1.33)	0.84
RR[3] (95% CI)	1.00	0.80 (0.59, 1.07)	0.84 (0.63, 1.13)	0.80 (0.59, 1.08)	0.99 (0.73, 1.34)	0.93
Eicosapentaenoic acid						
Median intake in subcohort (g/d)	0.00	0.01	0.02	0.04	0.08	
No. of cases	152	145	170	172	144	
RR[2] (95% CI)	1.00	1.18 (0.88, 1.56)	1.14 (0.87, 1.50)	1.23 (0.93, 1.62)	1.03 (0.78, 1.37)	0.63
RR[3] (95% CI)	1.00	1.15 (0.84, 1.58)	1.10 (0.82, 1.49)	1.22 (0.90, 1.65)	0.98 (0.72, 1.35)	0.87
Docosahexaenoic acid						
Median intake in subcohort (g/d)	0.01	0.03	0.05	0.08	0.14	
No. of cases	147	156	158	176	146	
RR[2] (95% CI)	1.00	1.11 (0.83, 1.47)	1.04 (0.78, 1.37)	1.20 (0.91, 1.58)	1.02 (0.77, 1.36)	0.62
RR[3] (95% CI)	1.00	1.10 (0.81, 1.51)	1.03 (0.76, 1.40)	1.21 (0.90, 1.64)	1.00 (0.72, 1.37)	0.70

[1] Reference category.
[2] Adjusted for age.
[3] Adjusted for age, history of benign breast disease, maternal breast cancer, breast cancer in one or more sisters, age at menarche, age at menopause, oral contraceptive use, parity, age at first childbirth, Quetelet index, education, alcohol use, current cigarette smoking, total energy intake, and total energy-adjusted fat intake.

TABLE 6
Rate ratios (RRs) and 95% CIs for breast cancer according to quintiles (Q) and categories of intake of milk products and meat in the Netherlands Cohort Study, 1986–1992

Exposure	Q1,C1[1]	Q2,C2	Q3,C3	Q4,C4	Q5	*P* for trend
Milk and milk products						
Median intake in subcohort (g/d)[2]	72	185	277	367	532	
No. of cases	170	148	165	142	158	
RR[3] (95% CI)	1.00	0.87 (0.66, 1.15)	0.91 (0.69, 1.19)	0.79 (0.60, 1.05)	0.93 (0.70, 1.22)	0.32
RR[4] (95% CI)	1.00	0.89 (0.66, 1.19)	0.87 (0.65, 1.17)	0.82 (0.60, 1.11)	0.91 (0.67, 1.24)	0.32
Whole milk and products						
Median intake in subcohort (g/d)[2]	0	21	53	107	232	
No. of cases	172	187	143	133	148	
RR[3] (95% CI)	1.00	1.07 (0.82, 1.39)	0.97 (0.73, 1.29)	0.83 (0.63, 1.10)	0.87 (0.66, 1.15)	0.04
RR[4] (95% CI)	1.00	1.10 (0.83, 1.47)	1.00 (0.73, 1.36)	0.88 (0.64, 1.21)	0.90 (0.66, 1.22)	0.12
Skim milk and products						
Median intake in subcohort (g/d)[2]	0	48	203			
No. of cases	350	187	246			
RR[3] (95% CI)	1.00	0.82 (0.66, 1.02)	1.01 (0.82, 1.24)			0.90
RR[4] (95% CI)	1.00	0.81 (0.64, 1.02)	1.04 (0.84, 1.30)			0.83
Fermented whole milk and products						
Median intake in subcohort (g/d)[2]	0	53				
No. of cases	587	196				
RR[3] (95% CI)	1.00	0.85 (0.70, 1.04)				0.05
RR[4] (95% CI)	1.00	0.88 (0.71, 1.10)				0.15
Fermented skim milk and products						
Median intake in subcohort (g/d)[2]	0	32	172			
No. of cases	200	272	311			
RR[3] (95% CI)	1.00	0.99 (0.78, 1.24)	0.98 (0.79, 1.23)			0.85
RR[4] (95% CI)	1.00	0.95 (0.74, 1.21)	0.89 (0.70, 1.14)			0.22
Cheese						
Median intake in subcohort (g/d)[2]	2	13	19	26	90	
No. of cases	181	121	167	196	118	
RR[3] (95% CI)	1.00	0.84 (0.63, 1.13)	0.90 (0.69, 1.17)	0.96 (0.74, 1.24)	1.00 (0.74, 1.34)	0.81
RR[4] (95% CI)	1.00	0.81 (0.60, 1.10)	0.88 (0.66, 1.17)	0.91 (0.69, 1.20)	0.94 (0.67, 1.31)	0.78
Butter						
Median intake in subcohort (g/d)[2]	0	13	31			
No. of cases	482	158	143			
RR[3] (95% CI)	1.00	1.14 (0.91, 1.43)	1.24 (0.97, 1.58)			0.02
RR[4] (95% CI)	1.00	1.11 (0.90, 1.54)	1.18 (0.90, 1.54)			0.08
Fresh meat						
Median intake in subcohort (g/d)[2]	45	73	91	107	145	
No. of cases	168	154	151	156	154	
RR[3] (95% CI)	1.00	0.98 (0.74, 1.29)	0.85 (0.65, 1.12)	1.02 (0.77, 1.34)	1.04 (0.79, 1.37)	0.69
RR[4] (95% CI)	1.00	0.95 (0.71, 1.27)	0.81 (0.61, 1.09)	1.00 (0.74, 1.35)	0.98 (0.73, 1.33)	1.00
Beef						
Median intake in subcohort (g/d)[2]	2	11	19	30	50	
No. of cases	140	158	169	139	177	
RR[3] (95% CI)	1.00	1.13 (0.85, 1.50)	1.13 (0.85, 1.49)	1.02 (0.76, 1.37)	1.29 (0.97, 1.70)	0.10
RR[4] (95% CI)	1.00	1.22 (0.90, 1.64)	1.10 (0.82, 1.49)	0.99 (0.72, 1.36)	1.23 (0.92, 1.66)	0.36
Pork						
Median intake in subcohort (g/d)[2]	3	18	31	45	72	
No. of cases	182	160	165	129	147	
RR[3] (95% CI)	1.00	0.96 (0.73, 1.26)	0.95 (0.73, 1.24)	0.77 (0.58, 1.02)	0.86 (0.66, 1.14)	0.04
RR[4] (95% CI)	1.00	0.93 (0.70, 1.25)	0.90 (0.67, 1.20)	0.77 (0.57, 1.04)	0.80 (0.60, 1.08)	0.02
Processed meat						
Median intake in subcohort (g/d)[2]	0	3	7	13		
No. of cases	128	330	189	136		
RR[3] (95% CI)	1.00	0.97 (0.75, 1.25)	0.95 (0.72, 1.27)	0.98 (0.72, 1.33)		0.83
RR[4] (95% CI)	1.00	0.93 (0.71, 1.23)	0.91 (0.68, 1.23)	0.93 (0.67, 1.29)		0.59

[1] Reference category.

[2] As raw weight.

[3] Adjusted for age.

[4] Adjusted for age, history of benign breast disease, maternal breast cancer, breast cancer in one or more sisters, age at menarche, age at menopause, oral contraceptive use, parity, age at first childbirth, Quetelet index, education, alcohol use, current cigarette smoking, and energy intake.

to the total evidence on CLA and breast cancer. No piece of evidence is conclusive but must be taken in the context of all the information available on CLA.

In the present analysis, RRs for TFAs were presented. TRANSFAIR data on fatty acids, used for the NLCS database, were based on concentrations before the end of 1995, when changes in the *trans* fatty acid content of manufactured consumer products, such as margarines, led to a decrease in the intake of TFAs in the Netherlands and other European countries. Because the follow-up reference period ended in December 1992, this change of TFA content will not have influenced the intakes of our subjects during follow-up. TFAs were positively associated with breast cancer incidence. Most prospective cohort studies addressing the relation between fat consumption and breast cancer did not mention results on TFAs, with the exception of a study in Seventh-day Adventists reporting no relation (8) and the Nurses' Health Study reporting an inverse association with TFAs (9). Two case-control studies (5, 6) did not find a positive association between TFA concentrations in adipose tissue and risk of breast cancer, nor have animal studies produced evidence for a contribution of TFAs to an elevated risk of breast cancer. A conclusion from a review in 1996 (56) was that there is little reason to assume that TFAs are related to risk of cancer in general or breast cancer in particular. An ecologic study that examined the correlation between breast cancer incidence and fatty acid status (as assessed in adipose tissue), however, did find a positive association with TFAs (57). Similar data on TFA status were also analyzed in a case-control study (7). In that study, too, TFAs were positively associated with breast cancer risk, in particular in population groups with the lowest linoleic acid status. A clinical study investigated whether the fatty acid composition of adipose tissue is linked to prognoses for women diagnosed with early-stage breast cancer (58). After an average follow-up of 7 y, no single fatty acid appeared to be associated with survival; however, this lack of association may be attributable to the small number of deaths in that study. On the other hand, the risk of positive lymph node findings was significantly higher for women with high concentrations of oleic acid or saturated fatty acids and lower for women with high TFA concentrations. None of these studies discriminated among the various TFA isomers or examined whether the TFAs were of animal or vegetable origin. One study showed that the correlation between intake and concentration in adipose tissue is much stronger for TFAs of vegetable than of animal origin (58).

In the present study, no association was observed between intake of linoleic acid and breast cancer incidence. In a review and meta-analysis, linoleic acid showed a negative association with breast cancer in 16 case-control studies but not in cohort studies (59). However, the results of the Nurses' Health Study show a significant inverse association (9). Also, no association was found for the fish $n-3$ PUFAs eicosapentaenoic acid and docosahexaenoic acid, whereas significant positive associations with these fatty acids originating from fish were found in the Nurses' Health Study.

Some general remarks about the present analysis on the NLCS have to be made. Loss to follow-up is the primary source of potential selection bias in prospective cohort studies. Given the high completeness of follow-up of the cases and subcohort person-years in the NLCS (34, 38) selection bias is unlikely. In prospective cohort studies, the potential problem of biased recall of past food intake (inherent in case-control studies) is avoided because dietary habits are reported before the disease is diagnosed. Although not likely, symptoms of breast cancer could have affected food consumption. However, reanalyses of data excluding (arbitrarily) incident cases in the first 2 y of follow-up did not change the results significantly (data not shown). The food-frequency questionnaire used was designed to measure fat intake, among other items. In the validation study, a correlation coefficient of 0.52 was calculated for total energy-adjusted fat intake, probably because of the relatively narrow range of fat intake in our population. The correlation coefficient for energy-adjusted polyunsaturated fat with a much wider range of intake was 0.75 (40). In addition to a validation study, 5 annually repeated measurements of the food-frequency questionnaire were conducted. From the results, it was concluded that the single measurement of intake of diet in the NLCS can characterize dietary habits for a period of $\geq$ 5 y (60). Furthermore, our study population consisted of older subjects (aged 55–69 y) who were chosen because, in general, they show more stable dietary habits than younger individuals (36). A disadvantage, however, is that potential effects of diet early in life cannot be studied. There are no reasons to assume that there is insufficient control of confounding in our analysis. We measured and controlled for the established risk factors of breast cancer. Finally, in the present study, multiple comparisons were made, which could have led to findings caused by chance. However, with 941 breast cancer cases, the power was large.

In conclusion, in this study no evidence was found for a protective effect of CLA on breast cancer incidence in postmenopausal women, as was suggested by animal experiments. If anything, the relation found was positive. The absence of a relation between breast cancer incidence and intake of energy or fat was in line with current opinion. Positive associations were also found for *trans* unsaturated fatty acids, saturated fatty acids, and *trans* vaccenic acid. Negative associations were found for monounsaturated and *cis* unsaturated fatty acids and the specific fatty acids oleic and linolenic acid.

We gratefully acknowledge the Regional Cancer Registries, the Dutch database of Pathology, and the National Health Care Information Center for providing incidence data. We also thank Sacha van de Crommert, Jolanda Nelissen, Conny de Zwart, Patricia Florax, and Willy van Dijk for expert help.

REFERENCES

1. Armstrong B, Doll R. Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. Int J Cancer 1975;15:617–31.
2. World Cancer Research Fund, American Institute for Cancer Research. Cancers, nutrition and food. Food, nutrition and the prevention of cancer: a global perspective. Washington, DC: World Cancer Research Fund, American Institute for Cancer Research, 1997.
3. Hunter DJ, Spiegelman D, Adami HO, et al. Cohort studies of fat intake and the risk of breast cancer—a pooled analysis. N Engl J Med 1996;334:356–61.
4. Howe GR, Hirohata T, Hislop TG, et al. Dietary factors and risk of breast cancer: combined analysis of 12 case-control studies. J Natl Cancer Inst 1990;82:561–9.
5. London SJ, Sacks FM, Stampfer MJ, et al. Fatty acid composition of the subcutaneous adipose tissue and risk of proliferative benign breast disease and breast cancer. J Natl Cancer Inst 1993;85:785–93.
6. Petrek JA, Hudgins LC, Levine B, Ho M, Hirsch J. Breast cancer risk and fatty acids in the breast and abdominal adipose tissues. J Natl Cancer Inst 1994;86:53–6.
7. Kohlmeier L, Simonsen N, van 't Veer P, et al. Adipose tissue trans fatty acids and breast cancer in the European Community Multicenter

Study on Antioxidants, Myocardial Infarction, and Breast Cancer. Cancer Epidemiol Biomarkers Prev 1997;6:705–10.
8. Mills PK, Beeson WL, Phillips RL, Fraser GE. Dietary habits and breast cancer incidence among Seventh-day Adventists. Cancer 1989; 64:582–90.
9. Holmes MD, Hunter DJ, Colditz GA, Stampfer MJ, Hankinson SE. Association of dietary intake of fat and fatty acids with risk of breast cancer. JAMA 1999;281:914–20.
10. Kritchevsky D. Antimutagenic and some other effects of conjugated linoleic acid. Br J Nutr 2000;83:459–65.
11. MacDonald HB. Conjugated linoleic acid and disease prevention: a review of current knowledge. J Am Coll Nutr 2000;19:111S–8S.
12. Fritsche J, Steinhart H. Amounts of conjugated linoleic acid (CLA) in German foods and evaluation of daily intake. Zeitschr Lebensm Unters Forsch 1998;206:77–82.
13. Banni S, Martin JC. Conjugated linoleic acid and metabolites. In: Sébédio JL, Christie WW, eds. Trans fatty acids in human nutrition. Dundee, Scotland: The Oily Press Ltd, 1998:261–302.
14. Belury MA. Conjugated dienoic linoleate: a polyunsaturated fatty acid with unique chemoprotective properties. Nutr Rev 1995;53:83–9.
15. Pariza MW, Ashoor SH, Chu FS, Lund DB. Effects of temperature and time on mutagen formation in pan-fried hamburger. Cancer Lett 1979;7:63–9.
16. Ha YL, Grimm NK, Pariza MW. Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid. Carcinogenesis 1987;8: 1881–7.
17. Cunningham DC, Harrison LY, Shultz TD. Proliferative responses of normal human mammary and MCF-7 breast cancer cells to linoleic acid, conjugated linoleic acid and eicosanoid synthesis inhibitors in culture. Anticancer Res 1997;17:197–203.
18. Shultz TD, Chew BP, Seaman WR. Differential stimulatory and inhibitory responses of human MCF-7 breast cancer cells to linoleic acid and conjugated linoleic acid in culture. Anticancer Res 1992;12: 2143–5.
19. Shultz TD, Chew BP, Seaman WR, Luedecke LO. Inhibitory effect of conjugated dienoic derivatives of linoleic acid and beta-carotene on the in vitro growth of human cancer cells. Cancer Lett 1992;63: 125–33.
20. Ip C, Singh M, Thompson HJ, Scimeca JA. Conjugated linoleic acid suppresses mammary carcinogenesis and proliferative activity of the mammary gland in the rat. Cancer Res 1994;54:1212–5.
21. Ip C, Scimeca JA. Conjugated linoleic acid and linoleic acid are distinctive modulators of mammary carcinogenesis. Nutr Cancer 1997; 27:131–5.
22. Ip C, Briggs SP, Haegele AD, Thompson HJ, Storkson J, Scimeca JA. The efficacy of conjugated linoleic acid in mammary cancer prevention is independent of the level or type of fat in the diet. Carcinogenesis 1996;17:1045–50.
23. Ip C, Jiang C, Thompson HJ, Scimeca JA. Retention of conjugated linoleic acid in the mammary gland is associated with tumor inhibition during the post-initiation phase of carcinogenesis. Carcinogenesis 1997;18:755–9.
24. Wong MW, Chew BP, Wong TS, Hosick HL, Boylston TD, Shultz TD. Effects of dietary conjugated linoleic acid on lymphocyte function and growth of mammary tumors in mice. Anticancer Res 1997;17: 987–93.
25. Durgam VR, Fernandes G. The growth inhibitory effect of conjugated linoleic acid on MCF- 7 cells is related to estrogen response system. Cancer Lett 1997;116:121–30.
26. Lin H, Boylston TD, Chang MJ, Luedecke LO, Shultz TD. Survey of the conjugated linoleic acid contents of dairy products. J Dairy Sci 1995;78:2358–65.
27. Precht D, Molkentin J. Trans-geometrical and positional isomers of linoleic acid including conjugated linoleic acid (CLA) in German milk and vegetable fats. Fett Lipid 1997;99:319–26.
28. Stanton C, Lawless F, Kjellmer G, et al. Dietary influences on bovine milk cis-9, trans-11-conjugated linoleic acid content. J Food Sci 1997;62:1083–6.
29. Maillard V, Bougnoux P, Ferrari P, et al. n−3 and n−6 fatty acids in breast adipose tissue and relative risk of breast cancer in a case-control study in Tours, France. Int J Cancer 2002;98:78–83.
30. Chajes V, Lavillonniere F, Ferrari P, et al. Conjugated linoleic acid content in breast adipose tissue is not associated with the relative risk of breast cancer in a population of French patients. Cancer Epidemiol Biomarkers Prev 2002;11:672–3.
31. Aro A, Mannisto S, Salminen I, Ovaskainen ML, Kataja V, Uusitupa M. Inverse association between dietary and serum conjugated linoleic acid and risk of breast cancer in postmenopausal women. Nutr Cancer 2000;38:151–7.
32. Lavillonniere F, Bougnoux P. Conjugated linoleic acid (CLA) and the risk of breast cancer. Adv Conjugated Linoleic Acid Res 1999;1: 276–82.
33. van Poppel G, van Erp-Baart MA, Leth T, et al. Trans fatty acids in foods in Europe: the TRANSFAIR study. J Food Comp Anal 1998; 11:112–36.
34. van den Brandt PA, van 't Veer P, Goldbohm RA, et al. A prospective cohort study on dietary fat and the risk of postmenopausal breast cancer. Cancer Res 1993;53:75–82.
35. de Deckere EA. Possible beneficial effect of fish and fish n−3 polyunsaturated fatty acids in breast and colorectal cancer. Eur J Cancer Prev 1999;8:213–21.
36. van den Brandt PA, Goldbohm RA, van 't Veer P, Volovics A, Hermus RJ, Sturmans F. A large-scale prospective cohort study on diet and cancer in The Netherlands. J Clin Epidemiol 1990;43:285–95.
37. van den Brandt PA, Schouten LJ, Goldbohm RA, Dorant E, Hunen PM. Development of a record linkage protocol for use in the Dutch Cancer Registry for Epidemiological Research. Int J Epidemiol 1990;19: 553–8.
38. Goldbohm RA, van den Brandt PA, Dorant E. Estimation of the coverage of Dutch municipalities by cancer registries and PALGA based on hospital discharge data. Tijdschr Soc Gezondheidsz 1994;72:80–4.
39. Voorlichtingsbureau voor de Voeding. NEVO table: Dutch Food Composition Table 1986–1987. The Hague: 1986.
40. Goldbohm RA, van den Brandt PA, Brants HA, et al. Validation of a dietary questionnaire used in a large-scale prospective cohort study on diet and cancer. Eur J Clin Nutr 1994;48:253–65.
41. Willett W. Implications of total energy intake for epidemiological analyses. In: Willett W, ed. Nutritional epidemiology. New York: Oxford University Press, 1990:245–71.
42. Prentice RL. A case-cohort design for epidemiologic studies and disease prevention. Biometrika 1986;73:1–11.
43. Stata Corp. STATA statistical software: release 6.0. College Station, TX: Stata Corporation, 1999.
44. Jiang J, Wolk A, Vessby B. Relation between the intake of milk fat and the occurrence of conjugated linoleic acid in human adipose tissue. Am J Clin Nutr 1999;70:21–7.
45. Ip C, Banni S, Angioni E, et al. Conjugated linoleic acid-enriched butter fat alters mammary gland morphogenesis and reduces cancer risk in rats. J Nutr 1999;129:2135–42.
46. Ip C, Scimeca JA, Thompson H. Effect of timing and duration of dietary conjugated linoleic acid on mammary cancer prevention. Nutr Cancer 1995;24:241–7.
47. Mills PK, Annegers JF, Phillips RL. Animal product consumption and subsequent fatal breast cancer risk among Seventh-day Adventists. Am J Epidemiol 1988;127:440–53.
48. Ursin G, Bjelke E, Heuch I, Vollset SE. Milk consumption and cancer incidence: a Norwegian prospective study. Br J Cancer 1990;61:456–9.
49. Knekt P, Jarvinen R, Seppanen R, Pukkala E, Aromaa A. Intake of dairy products and the risk of breast cancer. Br J Cancer 1996;73:687–91.
50. Byrne C, Ursin G, Ziegler RG. A comparison of food habit and food frequency data as predictors of breast cancer in the NHANES I/NHEFS cohort. J Nutr 1996;126:2757–64.

51. Toniolo P, Riboli E, Shore RE, Pasternack BS. Consumption of meat, animal products, protein, and fat and risk of breast cancer: a prospective cohort study in New York. Epidemiology 1994;5: 391–7.
52. Gaard M, Tretli S, Loken EB. Dietary fat and the risk of breast cancer: a prospective study of 25,892 Norwegian women. Int J Cancer 1995;63:13–7.
53. Boyd NF, Martin LJ, Noffel M, Lockwood GA, Trichler DL. A meta-analysis of studies of dietary fat and breast cancer risk. Br J Cancer 1993;68:627–36.
54. Knekt P, Jarvinen R. Intake of dairy products and breast cancer risk. Advances in conjugated linoleic acid research 1999;1:444–70.
55. Doyle E. Scientific forum explores CLA knowledge. Inform 1998; 9:69–73.
56. Ip C, Marshall JR. Trans fatty acids and cancer. Nutr Rev 1996;54: 138–45.
57. Bakker N, van 't Veer P, Zock PL. Adipose fatty acids and cancers of the breast, prostate and colon: an ecological study. EURAMIC Study Group. Int J Cancer 1997;72:587–91.
58. Petrek JA, Hudgins LC, Ho M, Bajorunas DR, Hirsch J. Fatty acid composition of adipose tissue, an indication of dietary fatty acids, and breast cancer prognosis. J Clin Oncol 1997;15:1377–84.
59. Zock PL, Katan MB. Linoleic acid intake and cancer risk: a review and meta-analysis. Am J Clin Nutr 1998;68:142–53.
60. Goldbohm RA, van 't Veer P, van den Brandt PA, et al. Reproducibility of a food frequency questionnaire and stability of dietary habits determined from five annually repeated measurements. Eur J Clin Nutr 1995;49:420–9.